The Impact of Flooding on Malarial Transmission within Roadway Communities in the Peruvian Amazon

by

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Thesis submitted in partial fulfillment of
the requirements for the degree of a Masters of Science in the Department of
Global Health in the Graduate School
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ABSTRACT

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Abstract

Severe flooding inundated the lowlands of Peru between 2011 and 2012. The rainfall and ensuing elevation in river levels coincided with a marked increase in human malaria. This study analyzes sampled *Anopheles* mosquitoes in twenty communities located on a new roadway system within the lowland Peruvian Amazon over a period of twelve months. 3,913 mosquitoes were captured and tested for *Plasmodium* sporozoite proteins via laboratory ELISA. Over eighty five percent (85%) of the samples were *Anopheles darlingi*. Half of the sampled communities reported at least one positive mosquito during the four rounds of collection. Transmission intensity was highly variable between communities, including the observation of spatial clustering of mosquito infectivity between communities near block sixteen (16). A negative binomial regression demonstrated smaller communities, closer to a river, were subject to higher mosquito densities. These same geographic sites did not exhibit statistically significant predicted rates of mosquito infectivity. Therefore, no additional conclusions can be made with regards to river distance on malaria burden. However, the observed heterogeneous patterns of malaria in collection sites could be driven by farm-to-market travel, previously explored in other investigations.

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1. Background

1.1 The History of Malaria Worldwide

Malaria remains at the forefront of the public health agenda, burdening over one hundred tropical and subtropical countries worldwide.[1,2] Half of the world's population live in areas where ongoing malaria transmission persists.[2] Equatorial countries in South East Asia, sub-Saharan Africa, and Latin America are the most burdened.

Millions of cases are reported every epidemiologic season within these countries, suggesting an upwards of 600,000 global deaths resulted from malaria in 2012.[2] Malaria is ubiquitous in tropical climates, posing serious implications on local economies and health systems. Families in these communities often lack access to appropriate health care facilities or health professionals. As a result, many community members forego treatment. Monitoring malaria remains one of the most promising tools in the step towards malaria eradication. Without proper surveillance and research on malaria, there cannot be a viable intervention.

Spanning the last century, a robust amount of funding has been channelled toward malaria eradication efforts. The introduction of DDT by the U.S. military in the forties was a valuable player in these campaigns. Different from preceding chemical agents, such as pyrethrum, DDT did not require weekly applications in households. In turn, local governments practically and proactively addressed malaria without the need

to spray rural communities on a weekly basis. Shortly after, the *World Health Organization* sought to leverage this technology by forming the *Global Malaria Eradication Program*, or GMEP, in 1955.

GMEP began to host DDT interventions in endemic regions by performing indoor residual spraying (IRS).[3] This diverged from traditional methods of destroying mosquito-breeding marshes and preventing mosquito bites in exposed populations.[3] The eradication campaign lasted until 1969, where lack of funding and re-emerging regional endemics signed the end of the GMEP. Concurrent to these efforts was the waning efficacy of antimalarials and chemotherapeutics on uncomplicated malaria.

As early as the fifties, essential chemotherapeutics demonstrated diminishing clearance times of *Plasmodium* in patients. Chloroquine resistance surfaced within both southeast Asia and South America in the late 1950s.[1] Decades before, South America reported the first case of quinine-resistant *Plasmodium*.[1] Several variables could contribute to drug-resistant strains: the extensive use of chemotherapeutic monotherapies, promotion of ineffective drugs, and genetic variability favouring resistance.[4] Outcomes the same, these variables culminate to shape the modern portfolio of efficacious chemotherapeutics today. Moreover, ensuring the sustained potency of chemotherapeutics call for more informed and effective delivery of resources to areas burdened by malaria

1.2 The Burden Asymmetry of Malaria in Northern Peru

The end of the *Global Malaria Eradication Program* in the 1960s was followed by sequestered DDT campaigns in 1988, reintroducing the predominant regional vector, *Anopheles darlingi*, back into Loreto.[5,6] The renewed presence of the *An. darlingi* in the lowlands was instrumental in the reintroduction of malaria in the region.[5] Since the sequestered campaigns, malaria dramatically rose, with cases in the Department of Loreto increasing from 1,500 before 1990 to over 100,000 cases in 1997[5]

Today, malaria disparately impacts the Department of Loreto, which houses roughly 90% of all the reported cases in 2012.[7] Even more surprising, Loreto comprises a quarter of the landmass but just three percent of the national population of Peru.[8,9] This unmistakable incongruence between population and malaria burden is startling, as almost the entire national malaria burden in Peru rests on a small subset of the population. These rural populations can be restricted in their access to health resources because of dense primary and secondary forest, limiting infrastructure development. For this reason, understanding the complex presence of malaria in the lower Amazon basin can assist in preparing local capacity to appropriately distribute resources to populations susceptible to transmission during flooding.

1.3 Record Flooding in the Department of Loreto

Starting in November 2011, La Nina weather patterns notably impacted the regional hydrology.[8] Flooding extended far beyond municipal boundaries of Iquitos, into surrounding communities along tributaries of the Amazon. The event inundated the local population between February and March 2012, affecting nearly 140,000 citizens.[10] In the subsequent months, malaria surveillance by the Ministry of Health reported double the number of malaria cases. This rise in malaria emphasizes the importance of flooding on anopheline breeding patterns and the observed elevation in the region's reported malaria.

1.4 The Role of Rivers on Malaria Transmission

The lower Amazon basin houses an expansive network of rivers and branching tributaries, extending throughout the region. Local hydrology has been observed as an important predictor of the variety and abundance of nearby anopheline populations.[11] Curiously, regional studies have found that community proximity to rivers does not always prompt higher mosquito populations.[12] In some cases, communities built at comparable distances to the same river demonstrated markedly different densities. Hiwat and colleagues commented on this complex ecologic predilection of anopheline species, as seemingly identical communities in the Maroni River in Suriname displayed

variable densities of anophelines, namely *A. darlingi*.[12] This incongruence in anopheline populations within seemingly identical communities could stem from river systems' various aquatic and ecologic properties at different locations. For example, bodies of water can change in flow rate, size, and surrounding ecology at different geospatial localities, either facilitating or hindering mosquito-breeding habitats. Moreover, larger populations of the *Anopheles darlingi* can lay the foundation for malaria transmission seen in past epidemics.[5] With this logic, communities at different segments of the same river system could experience different degrees of mosquito densities and possibly mosquito infectivity.

Table 1 GPS Coordinates for the Twenty Sampled Communities

Site	Block	Coding	Longitude	Latitude
Santo Tomas (2)	1	SAT2	-73.263	-3.688
Santo Tomas (1)	2	SAT1	-73.261	-3.676
Picuroyacu (2)	3	PIC2	-73.260	-3.658
Picuroyacu (1)	4	PIC1	-73.258	-3.639
San Antonio (3)	5	SAN3	-73.256	-3.623
San Antonio (2)	6	SAN2	-73.255	-3.605
San Antonio (1)	7	SAN1	-73.254	-3.584
18 de Enero	8	ENR	-73.249	-3.569
4 de Abril (4)	9	ABR4	-73.248	-3.553
4 de Abril (3)	10	ABR3	-73.241	-3.544
4 de Abril (2)	11	ABR2	-73.238	-3.530
4 de Abril (1)	12	ABR1	-73.234	-3.523
Ecosan	13	ECO	-73.218	-3.527
Tiwinza Manzur	14	TIW3	-73.205	-3.525
Tiwinza (2)	15	TIW2	-73.195	-3.524
Tiwinza (1)	16	TIW1	-73.179	-3.528
Albergue Santa Cruz	17	ASC	-73.165	-3.532
14 de Julio (2)	18	JUL2	-73.142	-3.531
14 de Julio (1)	19	JUL1	-73.130	-3.516
Puerto Alegre	20	PAL	-73.116	-3.509

1.5 Other Identified Determinants of Malaria in the Amazon Basin

The reintroduction of the *An. darlingi* and subsequent presence of malaria into the lowlands of Peru in the latter half of the 1900s highlights the importance of understanding vector behavior.[5] In particular, understanding which environmental and human determinants shape the presence of incriminated vectors, such as the *A. darlingi*. Some previously investigated factors influencing anopheline distribution include determinants such as local hydrology, land use, and mosquito biting behaviour. A

recent study on the effects of forest clearing on malaria identified mosquito densities in deforested areas as high as 278 times higher than forested areas.[13] Interestingly, these findings contrast the intuitive notion that larval habitats favor environments subject to less human influence. Another epidemiologic phenomenon in the region includes unseen levels of regional mosquito-infectivity within roughly twenty riverine communities north of Iquitos.[14] In these sampled communities, logging, fishing, and petrol are a key economic activities, whereby community members travel to variously populated and endemic locales. From these findings, it was hypothesized that these mobile labor populations could be promoting a higher prevalence of human and vector malaria observed in the local populations. Migrant laborers participate as channels by which malaria can be dispersed from higher transmission locales to lower transmission locales. In a sense, these migrant populations were vehicles for malaria when returning back to their respective community. A final study, conducted in the Brazilian Amazon, extensively studied the breeding patterns of several species of anopheline.[11] Upon completion of the study, several interesting observations were reported about the hydrological proclivity of the *An. darlingi* and its subsequent impact on mosquito populations within communities. Broadly put, different species of the anopheline mosquitoes expressed different hydrological 'preferences' for breeding. For example, the *An. Darlingi* expressed a partiality for permanent aquatic habitats, such as lakes and

ponds, while other species preferred more ephemeral aquatic habitats, such as pooled water in tires or drainage ditches.

1.6 Study Objective

This study is interested in understanding the transmission effects that flooding imposed on rural communities between 2011 and 2012. Specifically, understanding the elicited effect between river proximity and community size on mosquito densities and the presence of malaria, during intense periods of flooding in the lower Amazon basin.

2. Methods and Materials

2.1 Site Selection

The Bellavista Road is an infrastructure development project in the Maynas Provincial District north of Iquitos, Peru. The Bellavista road is one in a span of many infrastructure projects in the area, improving access to education, healthcare, and market sales for rural, peri-urban communities. The economy in Loreto is based on rich natural resources and primary forest, supporting lumber, agriculture, fishing, and petroleum industries.[5] Inhabitants of the roadway participate in several economic activities, predominantly agriculture and farming. Hence, farm-to-market travel is common. A total of twenty sites were sampled for anopheline mosquitoes once each month in June 2011 and between April and June 2012.

2.2 Sampling and Human Landing Collections (HLC)

The designated sampling method for collections was Human Landing Collections (HLC). During initial nightly collections, six trained field (three groups of two) workers exposed their legs for non-continuous hours, aspirating landed mosquitoes. Collections originally took place near households (if available) between 1800 and 0300, but this was later changed to 1800 to 0600 to sample *Anopheles* populations appearing in the early morning. Collection teams were offered malaria prophylaxis prior to collection. To ensure quality of collections, teams alternated hours

of periodic rest and breaks. Captured mosquitoes were placed with desiccant into a 1.5mL Eppendorf tubes labeled by location and hour for future identification. In addition, field teams recorded the corresponding temperature, relative humidity, rainfall, and vegetation of the surrounding site to potentially identify factors associated with heterogeneous vector densities. HLCs were used in human biting rates calculations, or the number of bites per person per unit time. Collections were conducted in weekly increments, once a month, between four non-consecutive months, July 2011, and April, May, and June 2012 and 2013 (data from 2013 are not included in this analysis). Unpredictable weather patterns dictated the location of collections within communities. As a result, collectors would either conduct inter- or intra-domiciliary collections during their stay.

2.3 Enzyme-Linked Immunosorbent Assay (ELISA)

Shortly after the completion of each collection period, mosquitoes were sent to a local insectary, NAMRU-6, Iquitos, for morphological species identification, and, subsequently, to the *Asociacion Benefica PRISMA* Tropical Research Lab, where CS-ELISA tests were employed (by species, date and location) to detect the presence of malaria sporozoites. The CS-ELISA has been described elsewhere.[15] Briefly, mosquitoes were ground into suspension and loaded onto high-affinity binding plates, coated with monoclonal antibodies. Monoclonal antibodies were used to distinguish parasite

species, *falciparum* and *vivax*, including two sub-variants, *P. vivax* VK210 and VK247. Plates were visually inspected against controls for fluoresced wells to denote 'positives' according to the 2009 CDC Sandwich ELISA protocol. Because of time and supply constraints, mosquitoes were pooled in groups of a maximum of three, according to the time of collection, date, location, and species. If the sum of mosquitoes was not divisible by three, and could not be pooled into groups of three evenly, pools of one, two, or four were added, as needed.

2.4 Quantifying Malaria Transmission

Calculating vector dynamics have been used in past eradication campaigns to assist in targeting areas at 'high risk' of transmission.[16] Table 2 summarizes the pertinent variables and respective equations used to quantify transmission in this study. Notably, each transmission value has several permutations and can be calculated a variety of different ways. For this reason, each variable below is defined according to its historical definition as well as the actual calculation utilized during this study. For example, instead of multiplying the mosquito density per person (m) and mosquito feeding rate (a), the human biting rate (a product of these values) was calculated by the number of captured mosquitoes per site divided by the total person-hours per site, which was typically sixteen hours – two collectors contributing eight hours each. This applied equation quantifies the number of bites per person in a given time. Moreover,

when conducting the laboratory ELISA, samples were pooled in groups of three within each ELISA well. The sporozoite rate (SPR) –defined as the proportion of infected mosquitoes - was calculated by dividing the number of positive mosquito wells morphologically identified per location by the total number of mosquitoes morphologically identified per location during each round of collections. Positives for both subvariants (VK210 and VK247) were combined during the calculation of sporozoite rates (SPR). Utilizing both of these transmission variables, we calculated the entomological inoculation rate (EIR), defined as the number of infected bites per person per unit time. To effectively mirror the malaria burden at the community-level, the EIR is defined as the number of infectious bites received per one hundred persons per eight hours each nightly collection. Calculating the EIR is a product of the calculated human biting rate (HBR) and the calculated sporozoite rate (SPR).

Table 2 Vector dynamics and respective equations used to quantify malaria transmission

Variable	Parameter	Equation
Z	Sporozoite Rate	$\frac{\text{Number of positive wells per site}}{\text{Number of total wells per site}}$
ma	Human Biting Rate	$\frac{\text{Number of captured mosquitoes per site}}{\text{Total person}-\text{hours per location per night}}$
maZ	Entomological Inoculation Rate	(Sporozoite Rate (Z) $\times$ Human Biting Rate (ma)) * 100

2.5 Statistical Analysis

Statistical analyses were conducted in STATA 12.0 (StataCorp LP, College Station, TX). Human biting rates (HBR) and sporozoite rates (SPR) were tested for significant monthly variation within revisited communities by using paired, two-tailed t-tests. Distance to the nearest river was used as a predictor utilizing Euclidean distances calculated from ArcGIS and FRAGSTATS 4.2v. in a negative binomial regression model to identify its relationship with HBR and SPR. Adjusted parameters included community size by number of households within a one-kilometer radius and date of collection.

3. Results

3.1 Mosquito Species Distribution

During four months of non-consecutive collections between 2011 and 2012 (July 2011, April-June 2012), 3,913 identifiable samples were captured. Of the near 4,000 collected samples, roughly 85% were identified as *Anopheles darlingi*. The remaining samples were identified as other unique species (seen in Table 8) or declared unidentifiable because of morphological damage: *oswaldoi* (OSW), *nuneztovari* (NUN), *triannulatus* (TRI), *forattini* (FOR), *mattogrossensis* (MAT), *rangeli* (RAN). Nineteen positive wells were identified of the 1,475 total wells analyzed by ELISA, of which only two were not with pooled *A. darlingi* – a single *A. oswaldoi* and *A. rangeli* well. Other

studies in the Brazilian Amazon demonstrated *Anopheles darlingi*, *An. oswaldoi* and *An. rangeli* as regional vectors for *Plasmodium vivax*.[11,17]

Not all of the captured mosquito samples were identified because of morphologic damage. The sporozoite rate (SPR) and entomological inoculation rate (EIR) only utilized samples that were capable of identification. The captured anopheline count was used in the calculations of HBR in Table 3 to accurately represent the observed mosquito density in the area at the time of collection. Mosquito densities were non-uniformly distributed between communities. The three communities with highest densities were 14 de Julio (1) (Block 18), Albergue Santa Cruz (Block 17), and Tiwinza (1) (Block 16), which comprised 1,728 mosquitoes, or 0.4357 (43.57%) of the captured mosquitoes. Moreover, the six least mosquito-dense communities contributed 221 samples (5.57%) of the total number of collected samples.

Table 3 Human biting rates and sporozoite rates for twenty sampled sites by date

	July 2011		April 2012		May 2012		June 2012	
Site	**HBR**	**SPR**	**HBR**	**SPR**	**HBR**	**SPR**	**HBR**	**SPR**
Puerto Alegre	3.313	0.000	2.563	0.000	2.250	0.000	1.313	0.000
14 de Julio (1)	8.625	0.000	8.625	0.000	5.625	0.000	0.688	0.200
14 de Julio (2)	4.563	0.000	8.750	0.064	1.875	0.091	0.000	0.000
Albergue Santa Cruz	2.875	0.000	34.250	0.000	5.500	0.000	0.063	0.000
Tiwinza (1)	25.125	0.000	20.313	0.000	13.250	0.014	1.875	0.077
Tiwinza (2)	2.750	0.000	11.813	0.000	0.625	0.000	0.125	-
Tiwinza Manzur	3.313	0.000	2.688	0.063	2.688	0.000	0.188	-
Ecosan	5.438	0.077	9.375	0.000	3.875	0.000	1.063	-
4 de Abril (1)	7.063	0.000	3.938	0.000	4.250	0.038	0.813	-
4 de Abril (2)	0.438	0.000	11.375	0.016	0.625	0.000	0.750	-
4 de Abril (3)	1.625	0.000	1.125	0.200	1.375	0.125	0.438	-
4 de Abril (4)	1.938	0.000	0.000	0.000	0.000	0.000	0.563	-
18 de Enero	0.000	0.000	0.875	0.000	0.375	0.000	0.125	-
San Antonio (1)	1.750	0.000	1.750	0.000	3.500	0.000	2.188	-
San Antonio (2)	3.188	0.000	7.188	0.000	3.688	0.000	1.750	-
San Antonio (3)	0.375	0.000	1.000	0.000	2.438	0.000	0.000	-
Picuroyacu (1)	0.438	0.000	2.500	0.000	0.063	0.000	2.000	-
Picuroyacu (2)	0.625	0.000	4.875	0.000	7.625	0.023	9.563	-
Santo Tomas (1)	0.688	0.000	4.625	0.000	5.063	0.133	8.438	-
Santo Tomas (2)	0.375	0.000	0.938	0.000	0.000	0.000	0.688	-

3.2 Human Biting Rate

Higher mosquito densities were observed near block sixteen (16), demonstrated in Appendix A, Figure 3. Human biting rate was the designated metric for mosquito density, and subsequently used to calculate community inoculation rates. Human biting rates ranged from 0.000 to 34.250 bites per person per hour within Albergue Santa Cruz.

Table 4 Negative binomial regression output for distance to the nearest river as a predictor of human biting rate in STATA 12.0

Negative binomial regression — Number of obs = 65
LR chi2(5) = 31.92
Dispersion = mean — Prob > chi2 = 0.0000
Log likelihood = -320.54724 — Pseudo R2 = 0.0474

OTOTL	Coef.	Std. Err.	z	P>|z|	[95% Conf.	Interval]
RIVER	-.0002719	.0000625	-4.35	0.000	-.0003944	-.0001494
SIZE	-.031323	.0075024	-4.18	0.000	-.0460273	-.0166187
MONTH						
2	.6490775	.3225878	2.01	0.044	.016817	1.281338
3	-.1170733	.3243711	-0.36	0.718	-.752829	.5186823
4	-1.697196	.5433765	-3.12	0.002	-2.762194	-.6321977
_cons	2.403936	.3300775	7.28	0.000	1.756996	3.050876
ln(HOURS)	1	(exposure)				
/lnalpha	.0146122	.176454			-.3312313	.3604557
alpha	1.014719	.1790513			.7180391	1.433983

Likelihood-ratio test of alpha=0: chibar2(01) = 2812.27 Prob>=chibar2 = 0.000

Three predictors were identified in the applied statistical model: Euclidean distance to the nearest river (meters), size of the community by number of households within a one-kilometer radius of sampling, and the date of collection. Table 4 and Table 5 include the STATA 12.0 outputs from a negative binomial regression. It is important to note that the X^2 in Table 4 is 2812.27, and a p-value less than 0.05, suggesting that the negative binomial model is, in fact, a better fit than the alternative Poisson model. Upon inspection, distance to the nearest river is negatively correlated with human biting rate (HBR). Meaning, for every Euclidean meter away from the nearest river, the predicted human biting rate declines by a rate of exp (-0.0002719), adjusting for date of collection and size of the community. For reference, the predicted human biting rate of a community 500 meters from the nearest river would be 9.66 bites per person per hour. In alignment, a sample site 4000 meters from the nearest river would have a predicted human biting rate (HBR) of 3.73 bites per person per hour. Size of the community has a similar relationship, demonstrating a predicted decline in the rate of human biting rate of exp (- 0.031323) for every additional household within a one-kilometer radius of sampling, adjusting for date of collection and distance to the nearest river (meters). The predicted human biting rate of a sample site containing forty (40) recorded households within a one-kilometer radius would be 3.161 bites per person per hour.

3.3 Mosquito Infectivity

The magnitude of *Plasmodia*-infected mosquitoes was highly variable between communities. Exactly half (10) of the sampled communities exhibited the presence of mosquito infectivity. Although *P. falciparum* was not identified in any of the sampled communities, it was present regionally according to confirmed human cases in the annual MOH reports during 2011 and 2012.[18] Five communities had two consecutive months of positives. Santo Tomas (1) was the only community with two corresponding positives on the same date of different subvariants, *P. vivax* VK210 and VK247.

Entomological inoculations ranged from 0 to 52.7. The community with the highest rate was 4 de Julio (2) with 52.7 infective bites per one hundred persons per night. Inoculation rates (EIR) were multiplied by one hundred to better reflect transmission intensity at a community level (infective bites per one hundred persons per night). The month of May displayed the highest overall inoculation rates between all twenty communities. Although, because of time constraints, the immunological analysis for June 2012 was incomplete, and only includes inoculation rates for blocks 16-20. The inoculation rates in June, for five analyzed blocks, appear comparable to transmission indices observed during the April and May of 2012.

Table 5 Negative binomial regression output for distance to the nearest river as a predictor of mosquito infectivity in STATA 12.0

Negative binomial regression — Number of obs = 65
LR chi2(5) = 5.59
Dispersion = mean — Prob > chi2 = 0.3484
Log likelihood = -40.309377 — Pseudo R2 = 0.0648

POS	Coef.	Std. Err.	z	P>|z|	[95% Conf.	Interval]
RIVER	-.0000233	.0001728	-0.13	0.893	-.000362	.0003155
SIZE	-.0350998	.0329796	-1.06	0.287	-.0997387	.0295391
MONTH						
2	1.138911	1.2277	0.93	0.354	-1.267337	3.545158
3	1.614529	1.219358	1.32	0.185	-.7753694	4.004427
4	2.780596	1.542753	1.80	0.071	-.2431442	5.804336
_cons	-5.108643	1.288105	-3.97	0.000	-7.633281	-2.584004
ln(WELL)	1	(exposure)				
/lnalpha	.503472	.6972403			-.8630939	1.870038
alpha	1.654456	1.153553			.4218549	6.488542

Likelihood-ratio test of alpha=0: chibar2(01) = 6.20 Prob>=chibar2 = 0.006

Corresponding with the aforementioned negative binomial model predicting human biting rate, mosquito infectivity was predicted through the same principle covariates. Seemingly, distance to the nearest river and size of the community by household were negatively related to the number of positively infected mosquitoes captured in the sample site. For every Euclidean meter farther from the nearest river predicted sporozoite rate (SPR) declined by a rate of exp (-.0000233), adjusting for size of

the community and date of collection. Furthermore, with every additional household within a one-kilometer radius of the sampling site predicted a decline in the sporozoite rate (SPR) by a rate of exp (-0.035099), adjusting for distance to the nearest river and date of collection. But, importantly, all three of the listed predictor variables, including month of collection, seen in Table 5, failed to demonstrate statistical significance with an accepted alpha = 0.05

Table 6 Entomological Inoculation Rate (EIR) by site

	July 2011			April 2012		
Site	*PK210*	*PK247*	**Total**	*PK210*	*PK247*	**Total**
Puerto Alegre	0.000	0.000	0.000	0.000	0.000	0.000
14 de Julio (1)	0.000	0.000	0.000	0.000	0.000	0.000
14 de Julio (2)	0.000	0.000	0.000	52.660	0.000	52.660
Albergue Santa Cruz	0.000	0.000	0.000	0.000	0.000	0.000
Tiwinza (1)	0.000	0.000	0.000	0.000	0.000	0.000
Tiwinza (2)	0.000	0.000	0.000	0.000	0.000	0.000
Tiwinza Manzur	0.000	0.000	0.000	6.641	0.000	6.641
Ecosan	18.269	0.000	18.269	0.000	0.000	0.000
4 de Abril (1)	0.000	0.000	0.000	0.000	0.000	0.000
4 de Abril (2)	0.000	0.000	0.000	18.347	0.000	18.347
4 de Abril (3)	0.000	0.000	0.000	31.250	0.000	31.250
4 de Abril (4)	0.000	0.000	0.000	0.000	0.000	0.000
18 de Enero	0.000	0.000	0.000	0.000	0.000	0.000
San Antonio (1)	0.000	0.000	0.000	0.000	0.000	0.000
San Antonio (2)	0.000	0.000	0.000	0.000	0.000	0.000
San Antonio (3)	0.000	0.000	0.000	0.000	0.000	0.000
Picuroyacu (1)	0.000	0.000	0.000	0.000	0.000	0.000
Picuroyacu (2)	0.000	0.000	0.000	0.000	0.000	0.000
Santo Tomas (1)	0.000	0.000	0.000	0.000	0.000	0.000
Santo Tomas (2)	0.000	0.000	0.000	0.000	0.000	0.000

Table 6 (continued)

	May 2012			June 2012		
Site	*PK210*	*PK247*	**Total**	*PK210*	*PK247*	**Total**
Puerto Alegre	0.000	0.000	0.000	0.000	0.000	0.000
14 de Julio (1)	0.000	0.000	0.000	13.750	0.000	13.750
14 de Julio (2)	0.000	16.477	16.477	0.000	0.000	0.000
Albergue Santa Cruz	0.000	0.000	0.000	0.000	0.000	0.000
Tiwinza (1)	18.125	0.000	18.125	13.942	0.000	13.942
Tiwinza (2)	0.000	0.000	0.000	-	-	-
Tiwinza Manzur	0.000	0.000	0.000	-	-	-
Ecosan	0.000	0.000	0.000	-	-	-
4 de Abril (1)	18.029	0.000	18.029	-	-	-
4 de Abril (2)	0.000	0.000	0.000	-	-	-
4 de Abril (3)	0.000	14.063	14.063	-	-	-
4 de Abril (4)	0.000	0.000	0.000	-	-	-
18 de Enero	0.000	0.000	0.000	-	-	-
San Antonio (1)	0.000	0.000	0.000	-	-	-
San Antonio (2)	0.000	0.000	0.000	-	-	-
San Antonio (3)	0.000	0.000	0.000	-	-	-
Picuroyacu (1)	0.000	0.000	0.000	-	-	-
Picuroyacu (2)	17.472	0.000	17.472	-	-	-
Santo Tomas (1)	16.458	49.375	65.833	-	-	-
Santo Tomas (2)	0.000	0.000	0.000	-	-	-

4. Discussion

4.1 Mosquito Species Distribution and Mosquito Density

Anopheline species were irregularly distributed amidst sampled communities. Upon observation, distance to the nearest river was a statistically significant predictor of mosquito density. Generally, the greater the distance the community was from a river, the lower the mosquito density – the predicted rate declining exp (-.0002719) for every Euclidean meter. In alignment, higher mosquito populations in riverine communities results has been linked to an ecological proclivity of the anopheline mosquito for more permanent aquatic breeding environments over more ephemeral aquatic environments.[11] Galardo et al. completed a vector study in Brazil suggesting that the dominant regional vector, *Anopheles darlingi,* favors permanent aquatic habitats, such as lakes and ponds.[11]

Community size was also observed as a predictor of site mosquito densities.. Broadly, the larger the community, the lower the mosquito density. Curiously, anthropophilic behavior of the *A. darlingi*, observed in previous investigations did not seem to impose a higher mosquito burden on more densely population communities.[14] These larger communities may still be at higher risk localized malaria transmission, but are more appropriately equipped with traditional public health resources, such as

insecticide-treated bed nets, which facilitate in reducing the entomological load or mosquito density in that locality.

4.2 Mosquito Infectivity

This study corroborated historic mosquito infectivity rates seen in the region.[14] Three mosquito species were identified carrying malaria sporozoites: *Anopheles darlingi*, *An. oswaldoi*, *An. rangeli*. All of the positively infected mosquito pools in this study were *Anopheles darlingi*, with the exception of two mosquitoes (*An. oswaldoi*/*An. rangeli*). Previous studies in the Amazon basin demonstrated compatible evidence for regional malaria vectors, identifying all three anopheline species as natural vectors for *Plasmodium vivax* by laboratory ELISA.[17] Many sample sites did not express positive *Anopheles* species. But human malaria cases did, in fact, persist in the region during the 2011/2012 epidemiologic seasons.

The negative binomial model in figure 2 did not render the selected covariates as statistically significant predictors of mosquito infectivity within sampled sites. Historically, environmental factors have certainly been implicated in the presence of anopheline populations.[11] And, yet, mosquito infectivity was not directly linked to communities' distances to the nearest river. Conversely, the low infection rate of

mosquitoes during collection periods may be supported by human mobility, observed in previous investigations.

In the region, Parker et al. hypothesized that human travel could disperse malaria from endemic areas to local communities.[14] In alignment, this phenomenon may also be present along communities on the roadway, as farm-to-market travel is a common economic activity. Human mobility could be driving local malaria cases on the Bellavista road but, with only the EIR, it is difficult to assess the stability of transmission in the area. Further investigation into the roadway's basic reproductive number, R_0, would provide more insight into the stability of transmission.

4.3 Summary and Concluding Remarks

Flooding in the Department of Loreto peaked in the months of April and May 2012. The ensuing HBR in the sampled communities declined, as the observed abundance of anopheline*s* tapered off in the months of May and June. A plausible explanation for this observed trend could stem from the variable effects of local hydrology on larval habitats. In some cases, the immediate ecological impact of flooding can, in fact, clear larval habitats. During this time, increased flow rates and river levels would likely not facilitate larval breeding. Yet, despite this hypothesis, smaller

communities closer to tributaries on the Bellavista road elicited higher mosquito densities during the 2011 and 2012 collections.

Aforementioned in previous sections, there are two critical phenomena that could drivers of the reported human malaria reported by the Peruvian ministry of health between the 2011 and 2012 epidemiologic seasons. Chiefly, smaller communities nearer to rivers carry a higher predicted human biting rate (HBR). The predicted abundance of the efficient vector, *A. darlingi*, within these smaller communities, places these communities at higher risk of malaria transmission. Rises in transmission intensity in the past also coincided with an elevated abundance of anopheline populations, specifically *A. darlingi*.[5] Hence, the ensuing potential of malaria transmission in these communities places rural community members at higher risk of transmission during the period of the flood.

Alternatively, it is possible that the presence of malaria could not be sustained during 2011 and 2012 epidemiologic seasons, and, instead, human cases of malaria were sustained through human mobility patterns. Parker and colleagues commented on similar environmental phenomena during preceding epidemiologic seasons in the Amazon basin. During Parker's study, transmission intensity was isolated within spatial foci, while neighboring collection sites reported very low levels of parasitaemia.[14]

Assisting in understanding this incongruent relationship between inadequate vector transmission and sustained clinical malaria, Parker et al. and Wesolowski et al also highlighted the role that human mobility has on the local endemic profile.[14,19] Correspondingly, the development of the Bellavista highway has been a nexus to otherwise inaccessible lowland locales, whereby farm-to-market travel bridges higher transmission areas with less susceptible communities on the Bellavista road. Speaking generally, this behavioral phenomenon could be responsible for sustaining elevated spatial isolates of transmission, while neighboring communities demonstrate sporozoite rates incapable of supporting transmission.

Further investigation in the region should be done to clarify the relationship between aquatic ecology and malaria patterns. Although research by Galardo et al. supported findings that anopheline populations inflate during extensive periods of rainfall, little regional evidence comprehensively bridges the relationship between flooding and parasitaemia in human populations.[11] To understand this relationship, future studies could conduct more frequent and community-specific sampling of mosquito and human populations between periods of intense rainfall. This would permit a more thorough understanding by highlighting the spatiotemporal trends of malaria following intense periods of rainfall. In addition, calculating additional

transmission indices, such as the basic reproductive number, R_0, could augment our understanding of the stability of transmission within these communities. And lastly, future investigations could study the biology of larval breeding sites to further understand the species-specific proclivities for various aquatic habitats.

Appendix A

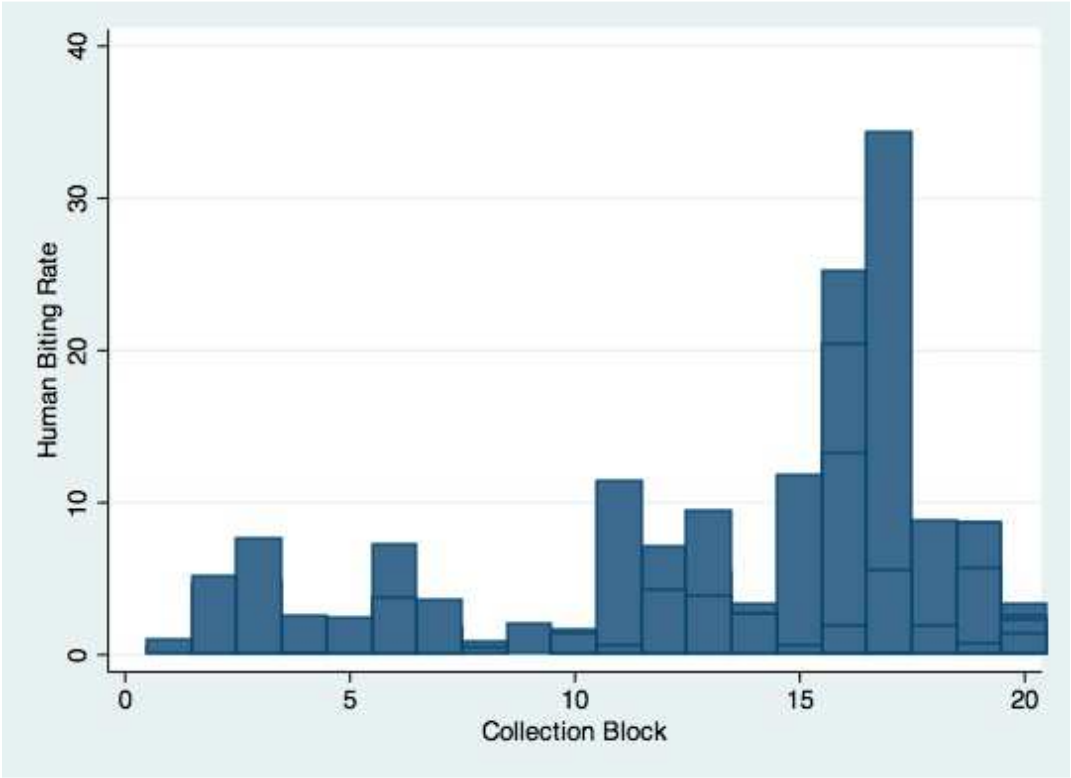

Figure 1 Human biting rates for all four rounds of collections by sample site

Appendix B

Table 7 Distance (meters) from communities to surrounding rivers

Sites	Block	River Distance
Santo Tomas (2)	1	0.00
Santo Tomas (1)	2	514.69
Picuroyacu (2)	3	1987.24
Picuroyacu (1)	4	2881.82
San Antonio (3)	5	4008.88
San Antonio (2)	6	5429.03
San Antonio (1)	7	7408.52
18 de Enero	8	8486.00
4 de Abril (4)	9	6660.81
4 de Abril (3)	10	5728.49
4 de Abril (2)	11	4510.65
4 de Abril (1)	12	3315.74
Ecosan	13	3848.71
Tiwinza Manzur	14	2920.65
Tiwinza (2)	15	2096.18
Tiwinza (1)	16	1798.12
Albergue Santa Cruz	17	1974.40
14 de Julio (2)	18	1922.61
14 de Julio (1)	19	326.03
Puerto Alegre	20	308.92

Appendix C

Table 8 The distribution of Anopheles among sampled sites

Site	Block	*DAR*	*OSW*	*SPP*	*NUN*	*TRI*	*FOR*	*MAT*	*RAN*	**Total**
SAT2	1	24	4	1	-	-	-	-	-	29
SAT1	2	72	42	6	11	12	1	-	-	144
PIC2	3	105	50	14	24	9	1	-	4	207
PIC1	4	38	4	2	-	-	-	-	-	44
SAN3	5	46	8	2	-	-	-	-	4	60
SAN2	6	168	37	3	-	2	-	1	7	218
SAN1	7	77	23	6	2	-	-	1	-	109
ENR	8	12	1	-	-	-	-	-	-	13
ABR4	9	15	-	2	-	-	-	-	-	17
ABR3	10	51	5	1	-	-	-	-	2	59
ABR2	11	195	-	-	-	-	-	-	-	195
ABR1	12	186	-	2	-	2	-	-	-	190
ECO	13	239	7	2	-	-	-	-	-	248
TIW3	14	92	9	10	-	-	-	-	-	111
TIW2	15	192	6	4	-	-	-	-	-	202
TIW1	16	669	25	56	-	-	-	-	-	750
ASC	17	633	4	4	-	-	1	-	-	642
JUL2	18	176	23	4	-	-	-	-	-	203
JUL1	19	289	25	21	-	2	1	-	-	338
PAL	20	108	12	8	5	1	-	-	-	134
Total		3387	285	148	42	28	4	2	17	3913
Percentage		86.56%	7.28%	3.78%	1.07%	0.72%	0.10%	0.05%	0.43%	100%

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